

C L A I M E S

1. A paste comprising bacteria-CWS which consists of a bacteria-CWS and an oil wherein the paste has a viscosity of 0.7 poise or less (25 °C).

2. The paste comprising bacteria-CWS according to claim 1 wherein the paste has a viscosity between 0.2 and 0.7 poise (25 °C).

3. The paste comprising bacteria-CWS according to claim 1 wherein the paste has a viscosity between 0.28 and 0.55 poise (25 °C).

4. The paste comprising bacteria-CWS according to any one of claims 1 to 3 wherein the bacteria-CWS is BCG-CWS.

5. The paste comprising bacteria-CWS according to any one of claims 1 to 4, wherein the oil is squalane.

6. The paste comprising bacteria-CWS, wherein the bacteria-CWS is BCG-CWS and wherein the paste comprises 6.6 g to 35.2 g of squalane per about 0.67 g of BCG-CWS.

7. A process for preparation of a paste comprising bacteria-CWS, which comprises the following steps:

(1) a step of mixing the bacteria-CWS and oils in an organic solvent used as a dispersion-aiding solvent; and

(2) a step of removing the organic solvent in (1) by distillation.

8. The process for preparation according to claim 7 wherein the organic solvent comprises a hydrocarbon solvent and a halogenated hydrocarbon solvent.

9. The process for preparation according to claim 8, wherein the organic solvent is a hydrocarbon solvent which comprises 5 to 20 %

(v/v) of an alcohol solvent.

10. The process for preparation according to claim 8 or 9, wherein the hydrocarbon solvent is heptane or hexane.

5 11. A paste comprising bacteria-CWS obtainable by the process for preparation according to any one of claims 7 to 10.

12. The paste according to claim 11 wherein the bacterium is BCG bacteria.

13. The paste according to any one of claims 7 to 12 wherein the oil is squalane.

10 14. An oil-in-water emulsion which comprises the paste comprising bacteria-CWS according to any one of claims 1 to 6 and 11 to 13, a surfactant, a stabilizer, and water.

15 15. The oil-in-water emulsion according to claim 14, which comprises 0.66 g to 3.35 g of the bacteria-CWS, and 0.4 wt% to 8 wt% of the oil per 2L of water.

16. The oil-in-water emulsion according to claim 14 or 15, wherein the stabilizer comprises 1 to 10 % mannitol.

20 17. The oil-in-water emulsion according to any one of claims 14 to 16, wherein the surfactant comprises 0.01 % to 3% polyethylenoxysorbitan fatty acid ester.

18. The oil-in-water emulsion according to claim 17, wherein the polyethylenoxysorbitan fatty acid ester is Tween 80.

19. The oil-in-water emulsion according to any one of claim 14 to 18, having the following properties:

25 (1) the particle diameter of an oil droplet of the emulsion is 0.2 to 30 μm ;

(2) the bacteria-CWS is encapsulated in the oil droplet, and is negative for reaction with lectin.

20. A process for preparation of the oil-in-water emulsion according to any one of claims 14 to 19, which comprises the following steps:

(1) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of claims 1 to 10 and 17 to 19, and an aqueous solution containing a surfactant at a temperature higher than the turbidity point; and

(2) a step of adding an aqueous solution containing a stabilizer for dilution.

21. The process for preparation according to claim 20 wherein the emulsification step in above step (1) comprises the following steps:

(3) a step of emulsifying a mixture comprising the paste comprising bacteria-CWS according to any one of claims 1 to 6 and 11 to 13, and an aqueous solution containing 0.02 % to 0.8 % of a surfactant (rough emulsification step); and

(4) a step of adding an aqueous solution containing a surfactant to the mixture of (3) to adjust the concentration of the surfactant, and vigorously stirring the mixture (complete emulsification).

22. A lyophilized formulation obtainable by lyophilizing the emulsion according to any one of claims 14 to 19.

23. The lyophilized formulation according to claim 22, wherein the emulsion is obtainable by the process according to claim 20 to 21.

24. An assembly of bacteria-CWS particles wherein the particle diameter is 0.15 to 6 μm in the particle size distribution.

25. The assembly of bacteria-CWS particles according to claim 24, wherein the particle size distribution shows a single peak, as well as D10%: 0.23 ± 0.05 and D90%: 0.60 ± 0.05 .

26. A process for preparation of the assembly of bacteria-CWS particles according to claim 24 or 25, which comprises dispersing the bacteria-CWS in a solvent containing an aliphatic hydrocarbon solvent.

27. The process according to claim 26, wherein the solvent is a mixture of an aliphatic hydrocarbon solvent and an alcohol solvent.

28. The process for preparation according to claim 27, wherein the solvent is a heptane containing 5 to 20 % ethanol.

29. A process for identification of species and/or strains of a bacterium from which bacteria-CWS is derived, which comprises the following steps:

(1) a step of separating and/or extracting the long-chain fatty acid contained in the bacteria-CWS to prepare a long-chain fatty acid fraction, and if necessary, converting the long-chain fatty acid in the long-chain fatty acid fraction into a derivative thereof;

(2) a step of determining the long-chain fatty acid or a derivative thereof in the long-chain fatty acid fraction of (1) by chromatography; and

(3) a step of identifying species and strains of a bacterium from which the bacteria-CWS is derived based on the results of determination (2).

30. The process according to claim 29 wherein step (1)

comprises a step of labeling the long-chain fatty acid in the long-chain fatty acid fraction to prepare a labeled long-chain fatty acid derivative;

31. A process for assay of the strength of a bacteria-CWS, which comprises the following steps:

5 (1) a step of separating and/or extracting the long-chain fatty acid contained in the bacteria-CWS to prepare a long-chain fatty acid fraction, and if necessary, converting the long-chain fatty acid in the long-chain fatty acid fraction into a derivative thereof;

10 (4) a step of determining the content of the long-chain fatty acid or a derivative thereof in the long-chain fatty acid fraction; and

(5) a step of evaluating for an immunopotentiating activity of the bacteria-CWS based on the results of determination (4).

32. The process according to claim 31, wherein step (1) determining the content of the long-chain fatty acid or a derivative thereof comprises a step of labeling the long-chain fatty acid in the long-chain fatty acid fraction to prepare a labeled long-chain fatty acid derivative.

33. The process according to claim 30 or 32, wherein a derivative of the long-chain fatty acid is a long-chain fatty acid ester.

20 34. The process according to any one of claims 29 to 33, wherein the bacteria are those of Mycobacterium or Nocardia.

35. The process according to claim 34, wherein the bacteria of Mycobacterium are those of BCG.

25 36. The process according to any one of claims 29 to 35, wherein the long-chain fatty acid is mycolic acid.

37. The paste comprising bacteria-CWS according to claims 1

to 6 and 11 to 13 which comprises an assembly of bacteria-CWS particles, wherein the particle diameter is from 0.1 μm to 20 μm , preferably from 0.15 to 6 μm , and more preferably 0.2 μm to 2 μm in the particle size distribution.

5 38. The paste comprising bacteria-CWS according to claim 37, wherein the assembly of bacteria-CWS particles exhibit a particle size distribution showing a single peak as well as D10%: 0.23 ± 0.05 and D90%: 0.60 ± 0.05 .

10 39. An oil-in-water emulsion which comprises the paste comprising bacteria-CWS according to claim 37 or 38, a surfactant, a stabilizer, and water.

40. A lyophilized formulation obtainable by lyophilizing the emulsion according to claim 39.

15 41. A pharmaceutical composition which consists of the emulsion according to any one of claims 14 to 19 and 39.